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(54) Title: INTERFERON-GAMMA ANTI-INFLAMMATORY METHODS, COMPOUNDS, AND PHARMACEUTICAL COMPOSITIONS

(57) Abstract

A method for the use of IFN-γ inhibitors for reducing inflammation of the eye induced by IFN-γ, and pharmaceutical compositions for such method of use are described.

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Interfer n-Gamma Anti-Inflammatory Methods, Compounds, and Pharmaceutical Compositions

Priority

This application claims priority to U.S. Patent Application Serial No. 08/630,371, filed April 10, 1996.

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Brief Summary of the Invention

Field of the Invention

The instant invention involves methods of treatment of virus-induced corneal inflammation and the preparation of pharmaceuticals and use of IFN-γ inhibitors in such methods. In one particular embodiment, the method provides for treatment with pharmaceutical compositions containing fusion proteins which consist of an interferon-gamma (IFN-γ) receptor fused to the Fc portion of a human immunoglobulin (IFN-γR-Ig). Such a construct is known as a type of immunoadhesin. In a broad aspect, the invention encompasses the treatment of any corneal infection in which IFN-γ plays a role in inflammation by inhibiting the biological effect of IFN-γ.

Background of the Invention

A frequent cause of corneal infection is herpes simplex virus (HSV), type 1, known as herpes simplex keratitis, or herpes simplex keratoconjunctivitis. Herpes Simplex Virus corneal infection (HSV keratitis) is a leading infectious cause of blindness world-wide. There are approximately 500,000 new cases of HSV keratitis reported annually in the United States. A substantial proportion of these cases go on to develop potentially blinding inflammation of the cornea. Keratitis, or inflammation of the cornea, can occur in many forms. Acute or chronic, superficial or deep (see for example *The Merck Manual,* 16th, Rathaway, N.J. 1992).

Dendritic keratitis, is one form of the herpes virus infection which has a characteristic branched lesion of the cornea. This may then develop into stromal keratitis, which appears to reflect an immunologically mediated inflammation response to the virus.

Another form can occur due to infection by congenital syphilis (interstitial keratitis) or due to the inability to close the eye lids. Less common is keratitis that is caused by bacterial (i.e. *pseudomonas*) or fungal infections. Acute conjunctivitis, an inflammation of the conjunctiva is usually caused by viruses, bacteria, or allergy. Keratitis, usually occurring unilaterally in one eye, produces opacities of the cornea, mild to severe irritation, tearing, and photophobia.

Because stromal keratitis is due to an inflammatory response to the virus, often occurring in the absence of replicating virus, antiviral drugs are often ineffective because the virus is not actively replicating. Thus, treatment of this form of HSV keratitis is limited to trying no treatment, to attempted treatment of the inflammation with corticosteroids. Treatment with corticosteroids in this case carries significant risk of exacerbation of the condition if replicating virus is actually present. While treatment with corticosteroids can reduce scarring and subsequent blindness by reducing inflammation, the resulting complications if replicating virus is present pose a far greater threat. Thus, a method of treating inflammation without any risk of exacerbation would be very desirable.

Standard treatment of acute keratitis due to herpes simplex virus consists of idoxuridine eye drops and ointment whose activity is limited mostly to DNA viruses, primarily herpes simplex and poxviruses. Treatment with idoxuridine is hampered by the ability of virus to readily develop resistance to the drug, both *in vitro* and in man. Epithelial infections respond best, but the results are less favorable when the stroma is involved. Other localized herpes virus infections, such as those caused by type 2 simplex virus and varicella-zoster do not respond to the drug. Alternatively, vidarabine ointment may be used, which is active *in vitro* against vaccinia virus, herpes simplex virus types 1 and 2, varicella-zoster virus, variola virus, rhabdoviruses, and some RNA tumor viruses. Vidarabine applied topically is as effective as idoxuridine for herpes simplex keratoconjuctivitis (See for example *Goodman & Gilman's*, 8th edition, The Pharmacological Basis of Disease, Pergamon Press 1990). References cited in this description are incorporated by reference in their entirety as to any and all pertinent parts.

Treating corneal inflammation with topical corticosteroids can result in significant and vision-threatening exacerbation of the corneal disease. Due to the risks associated with steroid treatment of this disease, some physicians prefer to treat only with topical antiviral drugs, which usually do not alter the effects of corneal inflammation. Standard treatment of recurrent herpetic keratitis consists of trifluridine which is active against herpes simplex virus types 1 and 2 (including thymidine kinase-deficient strains), cytomegalovirus, vaccinia, and some strains of adenovirus. While viral resistance has not been reported, trifluridine exhibits mutagenic, teratogenic, and antineoplastic activities in experimental

systems.

Due to the risk of blindness resulting from uncontrolled inflammation, some physicians treat corneal inflammation with topical corticosteroids, even in view of the possible significant and vision-threatening exacerbation of the corneal disease if replicating virus is present. Thus, while typically, topical corticosteroids are contraindicated in dendritic keratitis, they may be effective when used with an antiviral agent in later stromal or uveitic involvement. With long-term use of corticosteroids, increased intraocular pressure (may exacerbate glaucoma) is a possibility, and should be monitored, as well as having the lens examined periodically for cataracts.

The typical cellular response to a viral infection is the release of Type I interferons (IFN-α and IFN-β) by the target cells. These type I interferons serve to inhibit viral replication, inhibit cell proliferation, enhance the lytic function of natural killer (NK) cells, and modulate Major Histocompatibility Complex(MHC) molecule expression which combine to aid in the eradication of viral infected cells (see for example *Cellular and Molecular Immunology* Abbas et al., 2nd, W.B. Saunders Co., Philadelphia, 1994). In response to infection, T-cells are stimulated to release cytokines which will activate inflammatory cells. Gamma interferon (IFN-γ), a type II interferon is one of them, and shares many of the same properties as type I interferons but with additional functions. The IFN-γ receptor is not related structurally to the receptor families which respond to the type I interferons. IFN-γ is a potent activator of mononuclear phagocytes. Thus IFN-γ is the principal macrophage-activating factor (MAF) produced by the T-cell. Further, IFN-γ effects MHC regulation, promotes B-cell and T-cell differentiation, activates neutrophils, NK cells, activates endothelial cells, and augments the biologic actions of tumor necrosis factor (TNF). Thus IFN-γ plays a major role in activation and potentiation of the inflammatory response.

More recently, IFN-γ has been implicated as an essential component of certain inflammatory responses in the uveal tract. Inflammation of the uveal tract, uveitis, can be part of such syndromes as Ankylosing spondylitis, Reiter's syndrome, Juvenile rheumatoid arthritis (JRA), Pars planitis, Toxoplasmosis, Cytomegalovirus (CMV) infection, Acute retinal necrosis, Toxocariasis, Birdshot choroidopathy, and Histoplasmosis among others. Thus some forms of uveitis are thought to be of autoimmune origin. Inflammation from autoimmune disease can also be mediated by IFN-γ.

Kürschner, C., Garotta, G., and Dembié, Z. (1992) "Construction, Purification, and Characterization of New Interferon g (IFN-γ) Inhibitor Proteins," *Journal of Biological*

Chemistry **267(13)**: 9354-9360, describes three constructs which consist of the mouse interferon-γ receptor extracellular portion and constant domains of immunoglobulins (Ig), specifically mouse γ2a, mouse κ, and human γ3. The hybrid fusion proteins were expressed in the mouse myeloma cell line J558L and recovered from the cell culture supernatant. All three constructs inhibited the binding of radioactive mouse IFN-γ to its receptor on L1210 cells. The constructs were demonstrated to be biologically active *in vitro* as neutralizing the action of mouse IFN-γ in an anti-viral assay.

Kürschner, C.; Ozmen, L.; Garotta, G.; and Dembic, Z. (1992) "IFNγ Receptor-Ig Fusion Proteins: Half-Life, Immunogenicity, and in Vivo Activity," *Journal of Immunology* **149(12)**: 4096-4100, describes further work to demonstrate *in vivo* utility of the fusion protein constructs. Experiments were performed on a model system for SZ (streptozotoxin)-induced diabetes in mice. The test mice were injected with 200 μl *i.p.* of a solution of murine IFN-γ inhibitors.

Haak-Frendscho, M.; Marsters, S.A.; Chamow, S.M.; Peers, D.H.; Simpson, N.J.; and Ashkenazi, A. (1993) "Inhibition of interferon-γ by an interferon-γ receptor immunoadhesin," *Immunology* **79**: 594-599, describes the development of murine/human IFN-γR-IgG. This construct combines an IFN-γR from mouse with the hinge region and Fc portion of human IgG1. Experiments demonstrated that the fusion protein bound IFN-γ, inhibited IFN-γ anti-viral activity *in vitro,* and was able to block the activity of endogenous murine IFN-γ *in vivo* by modulating the immune response to infection by *Listeria monocytogenes*.

In the following patent documents and foreign patents or applications, workers have isolated and used the IFN-γ receptor protein and inhibitors of IFN-gamma. All U.S. patent documents and published foreign patents or applications subsequently referred to in this description are hereby incorporated by reference in their entirety as to any and all pertinent subject matter, and the references cited therein.

For example, US Patent 4,897,264 issued Jan. 30, 1990 to Novick et al., entitled: "Human interferon-γ specific receptor proteins - useful in preparation of antibodies for blocking binding to some body cells of interferon-γ."

US Patent 5,221,789 issued June 22, 1993 to Novick et al., entitled: "New interferon-γ receptor extracellular fragment - useful for protecting against deleterious effects of IFN-γ, e.g. autoimmune diseases."

EP application 369 413 published May 23, 1990 by Novick et al., entitled: "Proteins

which bind specifically to interferon gamma - e.g. for treating autoimmune disease, and new DNA sequences, vectors and transformed cells, and derived antibodies."

EP application 369 877 published May 23, 1990 by Novick et al., entitled: "Recombinant DNA encoding type I interferon receptor protein - modulates cellular response to interferon."

EP application 393 502 published Oct. 24, 1990 by Fountoulak et al., entitled: "Soluble interferon-gamma receptors - for treating auto-immune diseases, chronic inflammations, etc."

WO 91/16431, a PCT application published October 31, 1991 by Narula et al., entitled: "New soluble, truncated gamma-interferon receptors - used to treat auto-immune diseases e.g. rheumatoid arthritis, multiple sclerosis, Sjorgen's syndrome and lupus erythematosus."

WO 94/14467, a PCT application published July 7, 1994 by Ashkenazi and Ward, entitled: "Treatment of inflammatory bowel disease with IFN-gamma inhibitors."

Infectious agents such as HSV, that induce tissue-destructive inflammatory responses and which can infect and destroy cells are very difficult to treat. Completely blocking the body's defense mechanisms (i.e. the immune/inflammation response to the virus) with drugs such as corticosteroids prevents the tissue-destructive inflammatory response, but can permit the virus to replicate unchecked, and spread to other parts of the body. Presently treatment of ocular infections and inflammation is based on intervention of the broad spectrum of immune response functions, and do not focus on the specific effectors. Thus it would be very useful to have a method for treating inflammation of the eye that did not result in a general debilitation of the body's immune defenses. Specific intervention of the IFN related pathways, which result in the debilitating inflammation of the effected ocular tissues, would provide a safer and more effective treatment than that which is presently practiced and would be of great use.

The following descriptions of the instant invention are meant to be illustrative only, and are in no way limiting as to the scope of the instant invention. One with ordinary skill in the art would readily recognize the scope and breadth of the teachings of the instant specification and be able to practice the various embodiments of the instant invention.

Brief Summary of the Invention

An object of the instant invention is to treat viral infection of the eye, and effectively treat related inflammation of the eye that is potentially blinding, with out danger of exacerbating the viral infection. The instant invention provides for a useful method of

reducing IFN-γ induced inflammation of the eye comprising administering an effective anti-inflammatory amount of an ophthalmic pharmaceutical preparation which will inhibit the effects of IFN-γ. In a preferred embodiment the active agent is administered in a suitable pharmaceutical carrier subconjunctivally via direct injection. In another preferred embodiment the active agent is administered in a suitable pharmaceutical carrier via absorption after topical application.

In a most preferred embodiment, the method of the instant invention provides for administering a pharmaceutical composition which comprises an effective anti-inflammatory amount of an IFN-γR-Ig construct, in an ophthalmic pharmaceutical preparation, which include but are not limited to solutions, suspensions, drops, ointments, balms, and creams. In particular, the instant invention provides a method of reducing IFN-γ induced inflammation of the eye from a viral infection by interfering with the action of IFN-γ. The instant invention provides a method of treating keratitis, uveitis or conjunctivitis by reducing IFN-γ induced inflammation of the eye from an infection, comprising administering an effective anti-inflammatory amount of an IFN-γR-Ig construct in an ophthalmic pharmaceutical preparation directly to the eye, or systemically. In a preferred embodiment, the IFN-γR-Ig immunoadhesins are formed using human amino acid sequences for the protein components.

The instant invention provides for a method of reducing IFN-γ induced inflammation of the eye caused by an infective agent, or due to autoimmune disease. The instant invention provides for the treatment of inflammation that is the result of, but not limited to, viral, bacterial, fungal or parasitic infection. The instant invention provides for treatment with IFN-γ inhibitors such as IFN-γR-Ig immunoadhesins, IFN-γ Receptor protein, and humanized antibody binding domains specific for IFN-γ or IFN-γ Receptors. This treatment has clinical applications in all animals that are susceptible to corneal inflammation, in particular mammals. The instant invention embodies various modes of administration including but not limited to intravenous , intramuscular, intraperitoneal, subcutaneous, and subconjunctival administration via injection. In a preferred embodiment, administration is by localized administration to the eye, but can also be via intravenous administration which may be more suitable for more severe forms of infection.

It is also further contemplated that the method of the instant invention embodies combined therapeutic treatment with IFN-γ inhibitors, such as IFN-γR-Ig immunoadhesins, in combination with other anti-viral agents.

A further object of the instant invention is to provide useful pharmaceutical

compositions for the practice of the instant invention. The pharmaceutical compositions and methods of administration of the instant invention could encompass injectable solutions, topically applied ointments, or eye drops which will contain an effective anti-inflammatory dose of an IFN-γ inhibitor, such as immunoadhesin IFN-γR-Ig. The pharmaceutical compositions of the instant invention can also include an effective dose of an anti-viral agent, to treat keratitis or conjunctivitis causing viral agents.

Effective dosages in mammals can range from 0.01 μg to 100 mg of IFN-γR-Ig in local administration. In a preferred embodiment, an effective dose contains at least 17 μg of IFN-γR-Ig immunoadhesin construct. An effective human dose may be in the range of 0.1 - 10.5mg, in particular a dose of from 1.0 to 1.5 mg would be preferred. For systemic administration, an effective dose takes into account body weight and blood volume of the subject, as well as blood pressure and circulation rates. For example, dosages of 0.01 to 1000 μg per liter are possible, but most effective when the local concentration in the eye can be maintained at effectively inflammation inhibiting levels. A preferred dose in 20 - 30 g mice can range from 40 to 250 μg, therefore in humans, a corresponding dose can be calculated on the basis of body weight to be from 1.5 to 10 μg per kilogram body weight to 1.5 to 10 mg per kilogram body weight. Effective systemic administration can be calculated on the basis of amount of therapeutic agent per liter of blood volume as well.

The instant invention thus provides useful methods for treating inflammation of the eye, and useful pharmaceutical compositions which can reduce IFN induced inflammation with or without the addition of an antiviral agent.

Brief Description of the Drawings

The invention will be better understood from a consideration of the following detailed description and claims, taken in conjunction with the drawings, in which:

Figure 1A is a graph depicting the results of *in vivo* experiments on a murine model using *i.p.* administration describing Disease Incidence as a percentage of mice with inflammation on the day recorded. Figure 1B shows mean Disease severity in animals that did show infection/inflammation on the day recorded.

Figure 2A is a graph depicting the results of *in vivo* experiments on a murine model using subconjunctival injection administration describing Disease Incidence as a percentage of mice with inflammation on the day recorded. Figure 2B shows mean Disease severity in animals that did show infection/inflammation on the day recorded.

Detailed Description of the Inv ntion

The instant invention teaches effective compounds and method for inhibition of IFN-γ activity, greatly reducing inflammation of the eye associated with infection or autoimmune disease, and the use of such compounds for the preparation of suitable medicaments for use in the methods. Previously there has been no safe, and effective means for treating inflammation of the eye without the risk of exacerbating the viral infection or possibly triggering an autoimmune reaction. The method of the instant invention teaches the effective treatment of ocular inflammation by inhibiting the action of IFN-γ. The method of the instant invention is suitable for treatment of such inflammation, without the danger of exacerbating any existing viral infection that may be still present. The applicable compositions embodied by the instant invention inhibit the action of IFN-γ and include the use of specific IFN-γ inhibitors, such as immunoadhesin IFN-γR-Ig, IFN-γ receptor, isolated IFN-γ receptor protein, and/or humanized active binding domain of antibodies specific for IFN-γ or the IFN-γ receptor. It is also contemplated that chemical inhibitors of the IFN-γ receptor, i.e. chemical antagonists specific for the receptor, can also be used to reduce inflammation.

Previous work has demonstrated a murine model for ocular disease and inflammation (Hendricks et al., (1992) "IFN-γ and IL-2 are protective in the skin but pathogenic in the corneas of HSV-1-infected mice" *Journal of Immunology* 149:3023-3028). This work demonstrated that HSV-1 topical infection of mouse corneas led to corneal epithelial lesions about 2 days after infection, and usually heal by 4 days post infection. The eyes and skin around the eyes appeared normal until about 7 to 10 days post infection, when a corneal stromal opacity consisting of predominantly polymorphonuclear leukocyte (PMN) infiltrate and edema developed. The corneal stromal inflammation usually progresses to severe necrotizing keratitis by 21 days post infection. Studies correlated the action of T-lymphocytes with prevention of dissemination of the virus, but also generating tissue-destructive inflammatory response in the corneal stroma.

Intraperitoneal injections with 0.5mg of rat anti-mouse IL-2, IL-4 or IFN-γ monoclonal antibodies was evaluated for efficacy in reducing the incidence of stromal inflammation as graded on a scale of 0 to 4+ by double-blinded slit-lamp examination of subject animals. It was found that the rat monoclonal antibody to IFN-γ was effective in reducing, but not eliminating inflammation, when initiated at 1 day before (30% disease incidence) or day 6 after infection (45% disease incidence). Rat monoclonal antibody to IL-2 was only effective at reducing inflammation when administered at day 6 after infection (50% disease incidence), showing about 58% disease incidence when given one day prior to

infection.

Most unfortunately, treatment with monoclonal antibody to IL-2 or IFN-γ one day prior to infection resulted in significant exacerbation of skin disease associated with the infection. Treatment with anti-IFN-γ rat monoclonal antibodies one day prior to infection, while showing some reduction in disease incidence, the disease severity was still high, rated from 2 to 3.5 (on a scale of 4). Treatment with anti-IFN-γ rat monoclonal antibodies at day six after infection, while showing some reduction in disease incidence, the disease severity was still rated from 1.7 to 2 (on a scale of 4). The treatment with monoclonal anti-IL-2 at day six after infection showed comparable disease severity of from 1.75 to 2 (on a scale of 4).

Thus it would be very useful to have an effective method for treating inflammation of the eye associated with infection or autoimmune disease related IFN-γ bioactivity. An especially effective treatment is one that will not result in a general debilitation of the body's immune defenses, or will not trigger an immune response in the subject. Specific intervention of the IFN-γ related pathways, which result in the debilitating inflammation of the effected ocular tissues, would provide a safer and more effective treatment than that which is presently practiced and would be of great use. In particular, the use of compositions which specifically inhibit the IFN-γ bioactivity, which are easy to manufacture, constructed so that they have a reduced incidence of triggering immune clearance of the drug from the subject, or adverse immune reaction, and are relatively small molecules would be most desirable.

In order to more clearly illustrate certain embodiments of the instant invention, the following examples are presented as illustrations of particular embodiments of the instant invention.

Example 1

IFN-γ Inhibitors and Immunoadhesin IFN-γR-Ig

An IFN-γR-Ig was previously described in Haak-Frendscho et al., (1993). This immunoadhesin combined the extracellular portion of the murine IFN-γ receptor with the hinge and Fc portion of an IgG1 heavy chain. The practical advantages of producing an immunoadhesin as a means of generating recombinant soluble binding proteins includes the ease of purification, capture and detection via the IgG-Fc portion which acts as a handle. Further, the addition of an antibody heavy chain to a soluble receptor often results in greater avidity for the ligand, and the increase in size can lead to dramatic increase in the circulating half-life of a relatively small binding protein by avoiding the rapid clearance that occurs for

small proteins via the kidneys. Several forms of IFN-γ inhibitors have been described in International PCT publication WO 94/14467, published July 7, 1994.

Thus one type of specific construct suitable for use in the methods of the instant invention are those which feature at a minimum, the property of specifically binding to IFN-γ. This form of construct will act such that IFN-γ will be bound and effectively inactivated, or at least inhibited in its biological activity by the construct. Another form of construct would employ the property of specific binding to IFN-γ Receptor protein, such that it acts as an antagonist of IFN-γ activity. The constructs contemplated by the instant invention are suitable for use in liquid suspension, cream suspension, ointment suspension, and can be administered by, for example *i.p.*, *i.v.*, or topical administration. The pharmaceutical compositions suitable for use in the instant invention will provide for an effective therapeutic amount of construct in a suitable pharmaceutical carrier. In particular, the IFN-γR-Ig immunoadhesin construct can be provided in an effective amount of from .01 μg to 100 mg, where the concentration in the carrier is determined by the general parameters of solubility/suspend ability of the construct in the carrier, and the size of the administered dose (i.e. drop size, amount of ointment, amount of *i.v.* solution etc.). Where the administration is via *i.v.* solution, further calculation as to effective dose per body weight is determined and factored in to the amount and concentration administered as practiced in the art.

Example 2

Treatment *in vivo* with IFN-γR-Ig Immunoadhesin - *i.p.* administration

Experiments were done to determine if treatment with IFN-γR-Ig would effectively inhibit the development of corneal inflammation, and severity of disease in mice. After infection with Herpes Simplex Virus type 1 (HSV-1), mice were given an intraperitoneal injection of 250 μg of IFN-γR-Ig six days after HSV-1 corneal infection with 1×10^5 PFU (plaque forming units) of Herpes simplex virus type I (HSV-I). A second injection of 50 μg IFN-γR-Ig, was given 13 days after infection. The corneas of the mice were then examined on specific indicated days after infection with a slit-lamp biomicroscope and scored for the degree of corneal inflammation by an observer who was unaware of the treatment groups.

The treated mice exhibited a significantly lower incidence and severity of corneal inflammation, when compared with control mice injected with only saline. These results show that IFN-γ plays a critical role in the inflammatory response to HSV infection of the cornea, and that systemic treatment with IFN-γR-Ig is effective in treating localized HSV

corneal infection. There were no untoward effects detected due to the treatment, i.e. disseminated disease, obvious toxic effects, or any immune reaction to the administration.

Figure 1 is a graph of the results where the data in Figure 1 are presented as the incidence of corneal inflammation on the indicated day after infection with HSV-1 and severity of disease. Figure 1A illustrates disease incidence as a percentage of mice with inflammation in each group. (Square: PBS treatment; Triangle: IFN-γR-Ig treated). Disease incidence for control mice was over 50% by day 12, and remained high, while incidence in IFN-γR-Ig treated mice never surpassed 25%. Figure 1B illustrates mean disease severity that day, as rated on a scale of 1 to 4, with 4 being the most severe inflammation. Disease severity was calculated using only those mice with actual infection and inflammation. (Triangle: PBS; Diamond: IFN-γR-Ig). Disease severity in control mice ranged from 0.5 to 2.5 (on a scale of 4), however the course of the disease was consistently above 1.5 from day 12 after infection onwards. In treated animals, the disease severity never passed 1.5, consistently remaining at a low level.

Example 3

Treatment *in vivo* with IFN-γR-Ig Immunoadhesin - subconjunctival injection

Because systemic treatment, while appropriate for severe infections, may induce a generalized cellular immune compromise in the subject, further experiments were performed to demonstrate the effectiveness of localized administration.

Additional mice were first infected, with 1 x 10^5 PFU (plaque forming units) of Herpes simplex virus type I (HSV-I). A subconjunctival injection into the infected eye of 17 μg of the IFN-γR-Ig immunoadhesin in phosphate buffered saline (PBS) was done on the eighth day after infection with HSV-I, which is just prior to the onset of clinically apparent corneal inflammation. Treatment was continued, every other day, through day 16 post infection. A control group of mice were treated in the same manner with PBS only. The corneas of the mice were then examined on the indicated days after infection with a slit-lamp biomicroscope and scored for the degree of corneal inflammation by an observer who was unaware of the treatment groups.

The treated mice exhibited a significantly lower incidence and severity of corneal inflammation, when compared with control mice. It is important to note that in most cases where treatment was discontinued after day 16 post infection, the inflammation resolved completely by 22 days after infection and did not recur. Any mild inflammation that was noted in a few of the treated animals, was resolved with further continued treatment. Doses lower than 17μg were tried, but proved less effective. There were no untoward effects

detected due to the treatment, i.e. disseminated disease, obvious toxic effects, or any immune reaction to the administration.

These results demonstrate that localized subconjunctival treatment is effective in treating localized HSV corneal infection. Figure 2 is a graph of the results where the data is presented as the incidence of corneal inflammation on the indicated day after infection with HSV-1 and severity of disease. Figure 2A illustrates disease incidence as a percentage of mice with inflammation in each group on the day measured. (Square: PBS treatment; Triangle: IFN-γR-Ig treated). Disease incidence for control mice was over 70% by day 12, and remained high, while incidence in IFN-γR-Ig treated mice never surpassed 20%, except for one day, and all incidence of disease disappeared by day 20. Figure 2B illustrates mean disease severity as rated on a scale of 1 to 4, with 4 being the most severe inflammation on specific days after infection. Disease severity was calculated using only those mice with actual infection and inflammation. (Square: PBS; Triangle IFN-γR-Ig). Disease severity in control mice ranged from 1.5 to 3 (on a 4 point scale), while Disease severity in treated mice never went beyond 1 (on a scale of 4 being maximum severity).

For human administration, it can be estimated that the human eye is about 80 times larger than the mouse eye. Since 17μg appears to be near the lower limit of an effective dose in mice, a corresponding human dose may be in the range of 1 - 1.5mg. However since the mouse cornea occupies a much larger area of the eye, than in humans, an even lower dose may be effective in humans. Of course, higher dosages can be administered depending upon the severity of the inflammation.

Example 4

Topical Application of IFN-γR-Ig Immunoadhesin

Effective topical administration to the eye, would be another preferred route of administration in the least critical incidence of infection. Suspensions of the contemplated active agents, i.e. IFN-γR-Ig, could be prepared following standard industry guidelines for optical topical application via, for example, creams, ointments, or sterile drops.

An effective inflammation inhibiting amount of the active agent, i.e. IFN-γR-Ig, would be prepared in a formulation which takes into account the typical amount of the applied pharmaceutical preparation, i.e. cream, ointment, drops, and the efficiency of making highly concentrated suspensions of the active agent in such pharmaceutical carrier. One advantage of using the immunoadhesin over full size antibody is the higher effective concentration of active component possible in a pharmaceutical preparation. Size is particularly important when topical administration is contemplated, because smaller

molecules can be transported through the epithelial cells of the cornea, or may pass through the intercellular spaces more easily than full size antibody molecules.

Taking the data from the mouse animal model, where at least 17μg of IFN-γR-Ig needs to be applied. In practice, amounts of up to 1g of IFN-γR-Ig, or other active agent could be applied via this mode of topical administration, depending upon the rate and amount of uptake into the eye. In order to facilitate such uptake, liposomal, or other such lipophilic carriers can be utilized to increase transfer of the effective inhibitors into the eye through the epithelium. Preparation of more highly concentrated starting material will aid the formulation of effective topical solutions/suspensions.

Example 5

Humanized Antibody active sites to IFN-γ or to IFN-γ Receptor

The previous example demonstrates that the inhibition of the activity of IFN-γ leads to a greatly reduced incidence of inflammation in a murine model system. Therefore the administration of an effective amount of humanized antibody active site which is specific for IFN-γ or IFN-γ receptor will act in a similar fashion to inhibit the effect of IFN-γ in causing inflammation. In order to avoid adverse immune reaction to the administration of foreign protein into the human system, whole antibody is avoided, and only the active binding domain is preferred. To further reduce the potential for adverse reaction to the binding domain protein, humanization of the domain, by substituting surface exposed residues with corresponding human protein sequence residues is contemplated. Thus the use of IFN-γR-Ig circumvents the potential for adverse immune reaction by the subject by using only human, or known compatible protein amino acid sequences.

Modification of the active site domains of the monoclonal proteins, i.e. humanization, will make the administration of these proteins in accordance with the teachings of the instant invention possible. Further modification of the binding domains, including bifunctional linkage, single chain antibody construct, and coupling to other protein carriers can also be accomplished.

Example 6

Treatment In Combination with Anti-viral Agents

The method of treatment of the instant invention is ideal for use in combination with traditional anti-viral agents for treatment of viral infections of the eye in combination with reduction of inflammation. Pharmaceutical compositions of the instant invention can be made which would combine an effective amount of the active agent, i.e. IFN-γR-Ig, with an

effective amount of an anti-viral agent. For example, anti-viral agents which can be used for the treatment of viral infections of the eye, and the typical dosages used are summarized below:

Table 1

Target: Herpes Simplex Virus Type 1 Keratoconjunctivitis	
Trifluridine	One drop of a 1% solution every 2 hours
Vidarabine	A 1.25 cm ribbon of 3% ointment every 5 times daily
Idoxuridine	One drop of a 0.1% solution every 1 to 2 hours
Target: Cytomegalovirus Retinitis	
Ganciclovir	5mg/kg every 12 hours *i.v.*
Foscarnet	230mg/kg daily *i.v.*

It will be understood that the specification and examples are illustrative but not limiting of the present invention, and that other embodiments within the spirit and scope of the invention will suggest themselves to those skilled in the art.

We Claim:

1. The use of an IFN-γ inhibitor in the preparation of a medicament for reducing IFN-γ induced inflammation of the eye.

2. The use according to Claim 1 wherein the IFN-γ inhibitor comprises one or more of an IFN-γR-Ig immunoadhesin, an IFN-γ Receptor, a humanized anti-IFN-γ Receptor, an anti-IFN-γ Receptor antibody binding domain, or a humanized anti-IFN-γ Receptor antibody binding domain.

3. The use according to Claim 1 wherein the IFN-γ induced inflammation is due to an autoimmune disease, an infective agent, a virus, a bacteria, or a fungus.

4. The use according to Claim 1 whereby additionally administering an effective amount of an antiviral agent.

5. The use according to Claim 1 whereby the pharmaceutical is administered directly to the eye.

6. A pharmaceutical preparation for reducing IFN-γ induced inflammation of the eye comprising an effective anti-inflammatory amount of an IFN-γ inhibitor in a suitable pharmaceutical carrier.

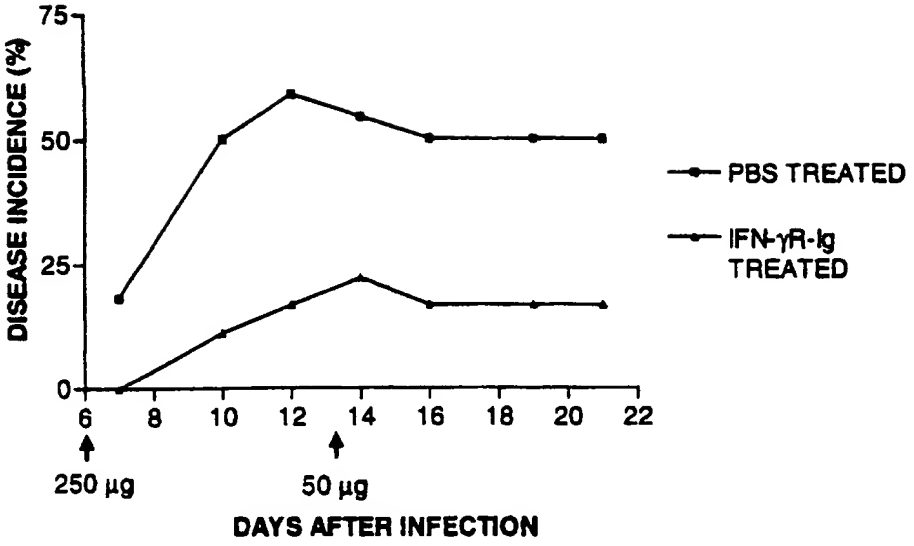
7. A pharmaceutical preparation as in Claim 6 where the IFN-γ inhibitor is an effective anti-inflammatory amount of an IFN-γR-Ig immunoadhesin, an IFN-γ Receptor, a humanized anti-IFN-γ Receptor, an anti-IFN-γ Receptor antibody binding domain, or a humanized anti-IFN-γ Receptor antibody binding domain.

8. A pharmaceutical preparation of Claim 6 which further comprises an effective virus inhibiting amount of an anti-viral agent.

9. A pharmaceutical preparation of Claim 8 where the anti-viral agent is selected from the group consisting of Vidarabine, Idoxuridine, Trifluridine, Ganciclovir, Acyclovir, and Foscarnet.

10. A pharmaceutical preparation of Claim 2 or 7 wherein the IFN-γR-Ig immunoadhesin is administered in a dose of from 0.01 μg to 100 mg, in a dose of at least 17 μg, in a dose of from 0.1 mg to 10.5 mg, 1 mg to 1.5 mg, in a dose of from 1 mg to 11 mg per Kg body weight, or in a dose of from 0.01 to 1000 μg per liter of blood volume.

Fig. 1A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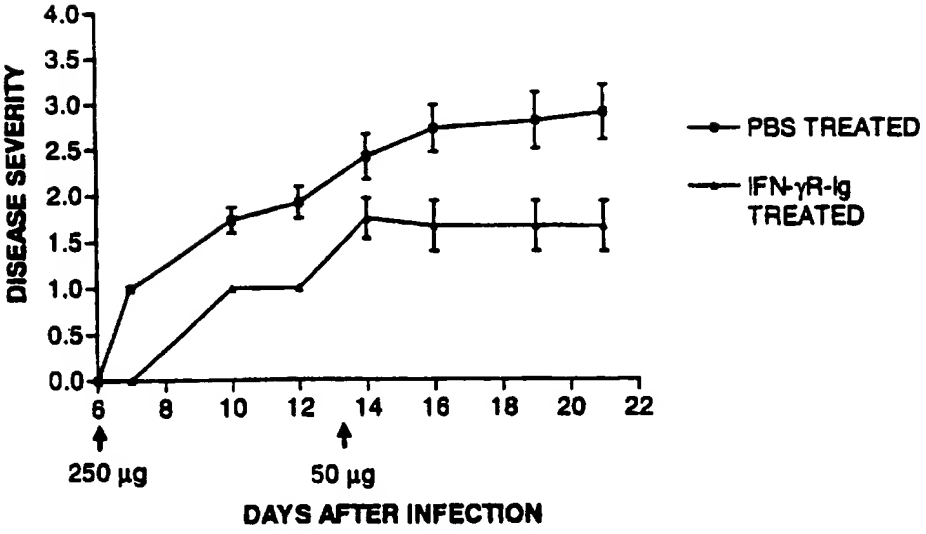
50 µg
DAYS AFTER INFECTION
PBS TREATED
IFN-γR-Ig TREATED

Fig. 1B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2.0
1.5
1.0
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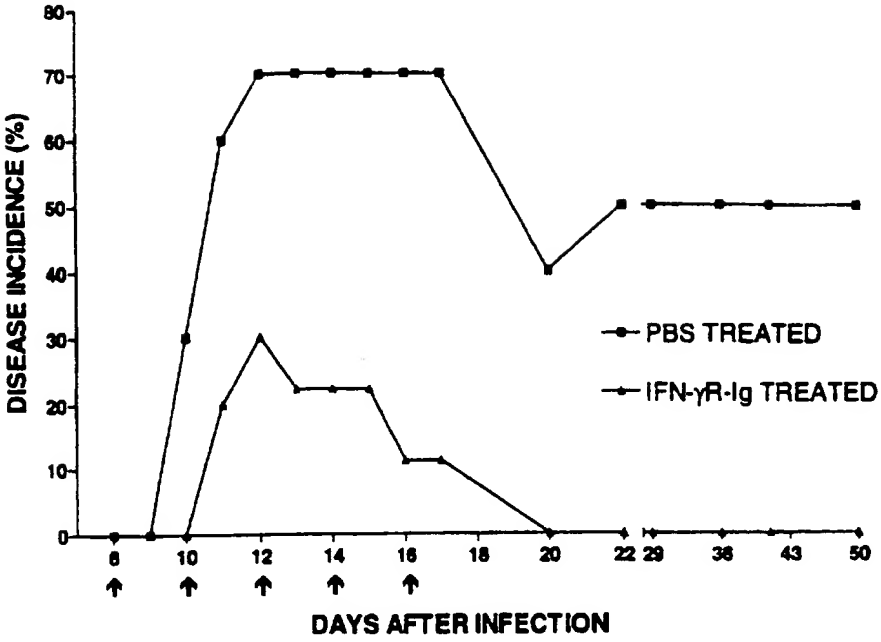
0.0
6
8
10
12
14
16
18
20
22
250 µg
50 µg
DAYS AFTER INFECTION
PBS TREATED
IFN-γR-Ig TREATED

Fig. 2A


DISEASE INCIDENCE (%)
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10
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14
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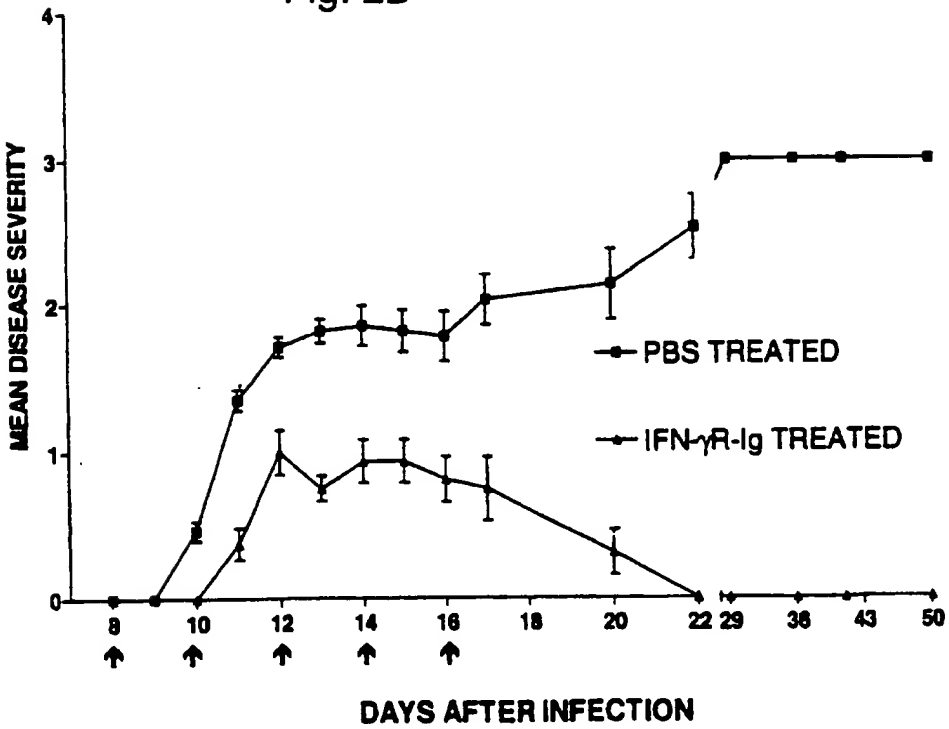
18
20
22
29
36
43
50
DAYS AFTER INFECTION
PBS TREATED
IFN-γR-Ig TREATED

Fig. 2B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22
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DAYS AFTER INFECTION
PBS TREATED
IFN-γR-Ig TREATED

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/US 97/05951

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K38/17 A61K39/395 //(A61K38/17,31:70),(A61K38/17,31:52), (A61K38/17,31:66)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOURNAL OF IMMUNOLOGY, vol. 149, no. 12, 15 December 1992, BALTIMORE US, pages 4096-4100, XP002035883 KÜRSCHNER C. ET AL.: "IFN-GAMMA RECEPTOR-Ig FUSION PROTEINS" cited in the application see the whole document ---	1-10
A	WO 94 14467 A (GENENTECH, INC.) 7 July 1994 cited in the application see the whole document --- -/--	1-10

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
23 July 1997	18.08.97
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Authorized officer Moreau, J

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/05951

C.(Continuation) DOCUMENTS C NSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	J IMMUNOL 149 (9). 1992. 3023-3028, XP002035884 HENDRICKS R L H ET AL: "IFN -GAMMA AND IL-2 ARE PROTECTIVE IN THE SKIN BUT PATHOLOGIC IN THE CORNEAS OF HSV-1-INFECTED MICE." see the whole document ---	1-10
A	THE 9TH INTERNATIONAL CONGRESS OF IMMUNOLOGY; SAN FRANCISCO, CALIFORNIA, USA, JULY 23-29, 1995, page 44 XP002035885 TANG Q ET AL: "Cytokine regulation of herpes simplex virus type-1 induced corneal inflammation." see abstract 255 -----	1-10

1

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter, nal Application No
PCT/US 97/05951

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		AU 5898894 A	19-07-94
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		EP 0695189 A	07-02-96
		JP 8504817 T	28-05-96
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